Rapid and Efficient Induction of Functional Astrocytes from Human Pluripotent Stem Cells

CURRENT STATUS: POSTED

Henrik Ahlenius
Lund Stem Cell Center

henrik.ahlenius@med.lu.se*Corresponding Author*

Isaac Canals
Lund Stem Cell Center

Aurélie Ginisty
Lund Stem Cell Center

Ella Quist
Lund Stem Cell Center

Raissa Timmerman
Lund Stem Cell Center

Jonas Fritze
Lund Stem Cell Center

Giedre Miskinyte
Lund Stem Cell Center

Emanuela Monni
Lund Stem Cell Center

Marita GrØnning Hansen
Lund Stem Cell Center

Isabel Hidalgo
Lund Stem Cell Center

David Bryder
Lund Stem Cell Center

Johan Bengzon
Lund Stem Cell Center

DOI:
10.1038/protex.2018.088
SUBJECT AREAS
Biological techniques *Neuroscience*
KEYWORDS
human astrocyte, stem cells, differentiation

Abstract

Current protocols for differentiation of human pluripotent stem cells to astrocytes are slow and inefficient, and characterization of the generated cells is incomplete. These shortcomings severely limit research on the biology of human astrocytes and their involvement in neurological disorders. Here we capitalized on recent transcription factor-driven methods to develop a novel protocol for astrocyte differentiation. We demonstrate that overexpression of two gliogenic transcription factors, Sox9 and Nfib, in human pluripotent stem cells rapidly and efficiently gives rise to a highly homogenous population of astrocytes as early as 7 days post-transduction. After 14 days, these induced astrocytes (iAs) exhibit molecular signatures and functional properties closely resembling those of adult human astrocytes. The iAs also recapitulate disease phenotype in a genome-engineered model of Alexander disease.

Our approach provides an easy, time-efficient, effective and scalable method that will provide new opportunities for studies on the role of human astrocytes in health and disease.

Reagents

Reagents:

DMEM/F-12 (ThermoFisher Scientific, cat. no. 31330038)

Neurobasal (ThermoFisher Scientific, cat. no. 21103049)

FBS (ThermoFisher Scientific, cat. no. 10082147)

N2 supplement (ThermoFisher Scientific, cat. no. 17502001)

B27 supplement (ThermoFisher Scientific, cat. no. 17504001)

NEAA (ThermoFisher Scientific, cat. no. 11140035)

Glutamax (ThermoFisher Scientific, cat. no. 35050061)

Sodium Pyruvate (ThermoFisher Scientific, cat. no. 11360070)

bFGF (Peprotech, cat. no. 100-18B)

CNTF (Peprotech, cat. no. 450-13)

BMP4 (Peprotech, cat. no. 120-05ET)

dbcAMP (Sigma-Aldrich, cat. no. D0627-25MG)

N-acetyl-cysteine (Sigma-Aldrich, cat. no. A8199-10G)

Heparin-binding EGF-like growth factor (Sigma-Aldrich, cat. no. E4643-50UG)

Polybrene (Sigma-Aldrich, cat. no. TR-1003-G)

Doxycycline (Sigma-Aldrich, cat. no. D9891-100UG)

Accutase (ThermoFisher Scientific, cat. no. A1110501)

Matrigel (Corning, cat. no. 354230)

Rock inhibitor (StemCell Technologies, cat. no. Y-27632)

Thiazovivin (StemCell Technologies, cat. no. 72252)

Puromycin (ThermoFisher Scientific, cat. no. A1113803)

Hygromycin (ThermoFisher Scientific, cat. no. 10687010)

Plasmids:

GFAP::GFP (Gift from Chun-Li Zhang, University of Texas Southwestern)

pMD2.G (Addgene #12259)

pRSV-Rev (Addgene #12253)

pMDLg/pRRE (Addgene #12251)

M2-rtTA (Addgene #20342)

tetO-Sox9-Puro (generated in this work)

tetO.Nfia.Blast (generated in this work)

tetO.Nfib.Hygro (generated in this work)

Reagent setup:

CaCl2: Dissolve at 2.5M in sterile water. Aliquot and store at −20°C.

Doxycycline: 25 mg/mL dissolved in water (10000x stock). Sterilize with a 0.22 μm filter and store at -20°C. Protect from light.

CNTF: Reconstitute at 10 μg/ml in sterile 10 mM sodium phosphate containing 0.1% BSA. Aliquot and store at -20°C.

bFGF: Reconstitute at 1 mg/ml in sterile 5mM Tris, pH 7.6 containing 0.1% BSA. Aliquot and store at -20°C.

BMP4: Reconstitute at 10 µg/ml in sterile 4 mM HCl containing 0.1% BSA. Aliquot and store at -20°C.

dbcAMP: Reconstitute at 100 mg/ml in sterile water. Aliquot and store at -20°C. Protect from light.

N-acetyl-cysteine: Reconstitute at 50 mg/ml in sterile water. Aliquot and store at -20°C.

Heparin-binding EGF-like growth factor: Reconstitute at 50 µg/ml in sterile PBS containing 0.1% BSA. Aliquot and store at -20°C.

Cell culture medium:

Expansion Medium:

DMEM/F-12

10% FBS

1% N2 supplement

1% Glutamax

FGF Medium:

Neurobasal

2% B27 supplement

1% NEAA

1% Glutamax

1% FBS

8 ng/ml FGF

5 ng/ml CNTF

10 ng/ml BMP4

Maturation Medium:

1:1 DMEM/F-12 and Neurobasal

1% N2

1% Sodium Pyruvate

1% Glutamax

5 µg/ml N-acetyl-cysteine

5 ng/ml heparin-binding EGF-like growth factor

10 ng/ml CNTF

10 ng/ml BMP4

500 µg/ml dbcAMP

Equipment

Incubator BBD 6220 (ThermoFisher Scientific)

Centrifuge Rotina 420R (Hettich Lab Technology)

Water Bath GD100 (Grant Scientific)

Ultracentrifuge Beckman Optima L-100K (Beckman Coulter)

Procedure

Lentiviral production:

Lentiviruses were produced in HEK 293T cells by co-transfecting pMD2.G, pRSV-Rev and pMDLg/pRRE helper vectors together with the vector for one transcription factor using 2.5 M CaCl2. 75 µg of transcription factor plasmids was used together with 30 µg of PMDLg/pRRE, 22 µg of pMD2.G and 15 µg of pRSV-Rev plasmids for two T175 flasks. Medium was changed 16 hours after transfection and viruses were harvested 48 hours after transfection, pelleted by centrifugation (20,000 x g for 2 hours at 4°C) resuspended in 100 µl DMEM, aliquoted and kept at -80°C.

iAs generation:

Day -2

Dissociate hESC or hiPSC with Accutase and replate 5x105 cells in Matrigel-coated 6-well plates with mTeSR1 containing 10 µM Rock inhibitor.

Day -1

Aspire medium and add 2 ml of fresh mTeSR1 containing 8 µg/ml of Polybrene per well. Add 1 µl of each virus per well.

Day 0

Aspire medium and add 2 ml of fresh mTeSR1 containing 2.5 µg/ml of Doxycycline per well.

Days 1 and 2

Aspire medium and add 2 ml of Expansion Medium containing 2.5 µg/ml of Doxycycline, 1.25 µg/ml of Puromycin and 200 µg/ml of Hygromycin per well.

Day 3

Aspire medium and add 2 ml of 3:4 of Expansion Medium and 1:4 of FGF Medium containing 2.5 µg/ml of Doxycycline and 200 µg/ml of Hygromycin per well.

Day 4

Aspire medium and add 2 ml of 1:1 of Expansion Medium and FGF Medium containing 2.5 µg/ml of Doxycycline and 200 µg/ml of Hygromycin per well.

Day 5

Aspire medium and add 2 ml of 1:4 of Expansion Medium and 3:4 of FGF Medium containing 2.5 µg/ml of Doxycycline and 200 µg/ml of Hygromycin per well.

Day 6

Aspire medium and add 2 ml of FGF Medium containing 2.5 µg/ml of Doxycycline.

Day 7

Dissociate cells with Accutase and pellet at 300g for 5 min. Replate cells in Matrigel-coated coverslips, petri dishes or wells according to desired output. Use the necessary amount of FGF Medium containing 2.5 µg/ml of Doxycycline.

Day 8

Aspire medium and add fresh FGF Medium containing 2.5 µg/ml of Doxycycline.

Day 10

Aspire half of the medium and add the same amount of Maturation Medium containing 2.5 µg/ml of Doxycycline. From here, change half of the medium every 2-3 days.